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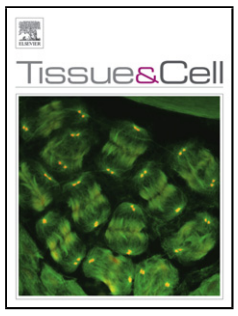

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In vitro-microenvironment directs preconditioning of human chorion derived MSC promoting differentiation of OPC-like cells

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Highlights

- Human chorion MSC secrete cytokines, survival and growth factors
- MSC conditioned with SRM express early stem cell markers
- SRM condition increases cell proliferation and migration of MSC
- Optimized MSC may support neuroregeneration

Abstract:

The loss of oligodendrocyte progenitor cells (OPC) is a hallmark of perinatal brain injury. Our aim was to develop an in vitro culture condition for human chorion-derived mesenchymal stem cells (MSC) that enhances their stem cell properties and their capability to differentiate towards OPC-like cells. MSC were grown either in serum replacement medium (SRM) or serum-containing medium (SM) and tested for their morphology, proliferation, secretome, migration, protein expression and differentiation into OPC-like cells. MSC cultured in SRM condition have distinct morphology/protein expression profile, increased cell proliferation/migration and capacity to differentiate into OPC-like cells.

Keywords

placenta; stem cells; mesenchymal stem cells; neurogenic differentiation; extracellular matrix; culture conditions; chorion; OPC; microenvironment.

Introduction

In recent years, mesenchymal stem cells (MSC) have been considered as a putative source of cells for regenerative approaches and stem cell grafting. They are capable of differentiating into cartilage, bone, adipose tissue, skin, liver, muscle and brain cells (Ullah I et all.,2015). MSC have been isolated from different tissues including the placenta (Portmann-Lanz et al., 2006). The human placenta is a feto-maternal entity that consists of three layers: decidua (maternal), chorion (fetal) and amnion (fetal). MSC obtained from the placental tissue can differentiate into various lineages including neural cells (Portmann-Lanz et al., 2010).

The stem cell graft's efficiency to migrate, home, integrate, survive, proliferate and differentiate into the appropriate cell types needs to be improved. The regenerative processes in cell transplantation paradigms greatly rely on the release of trophic factors that support cell activities (of either differentiated cells or resident stem cells) (Schoeberlein et al., 2011). Despite the advancements in stem cell technology, there is a high necessity to further unravel the properties of MSC and mechanisms of regeneration. Thus, optimization of stem cell techniques is essential to overcome such difficulties.

We have previously shown that the neurogenic potential of chorion MSC was higher compared to MSC from both bone marrow (BM) and amnion (Portmann-Lanz et al.,

2006). In this study, human chorion MSC were exposed to different conditions (microenvironment) that were previously published, grown on serum media (SM) (Portmann-Lanz et al., 2006, Battula et al., 2007, 2008)) and in serum replacement medium (SRM)(Battula et al., 2007, 2008). In the present study, we investigated whether or not changing the microenvironment would improve MSC properties and promote differentiation into OPC like-cells.

Materials and Methods

Isolation and culture of human chorion-derived MSC

The Institutional Review Board approved all experiments. Written consent was obtained from patients (Department of Obstetrics, The University Hospital Bern) before sampling of placental tissue. MSC were isolated from the chorion, which is the fetal part of placenta from normal term as described (Portmann-Lanz et al., 2006) . MSC were cultured until passage 4 using serum medium (SM: Dulbecco's Modified Eagle's Medium (DMEM)/F12, 10% fetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 1x GlutaMAX™ [Life Technologies, Carlsbad, CA, USA]), and expanded at 37°C, 5% CO_2. At passage 5, two different culture conditions were tested: $5x10^5$ cells were grown in uncoated culture flasks (150cm^2) with 20ml conventional culture medium (SM) or on tissue culture flasks coated with 0.1 % gelatin in 20ml human ESC medium (knockout DMEM, Sigma-Aldrich, St. Louis, MO, USA, 20% knockout serum replacement, Life Technologies; 1x GlutaMAX™, 0.1 mM β-mercaptoethanol, Sigma-Aldrich; 1% non-essential amino acids, 5 ng/ml human basic fibroblast growth factor, bFGF, PeproTech, Rocky Hill, NJ, USA) (SRM) (Battula et al., 2007; Xu C et al., 2001). To keep up the bFGF levels, 2 ml of used medium (SRM) was replaced with fresh

medium every second day. Thus, MSC were grown until passage 4, preconditioned at passage 5 and detached after reaching 90% confluency to carry out the following analyses.

Flow cytometry

Cells grown in the two experimental conditions were analyzed by flow cytometry for extracellular markers at passage 5: CD105, CD90, CD73, CD45, CD34, CD14 and HLA-DR (Portmann-Lanz et al., 2006). MSC at passage 5 were trypsinized, resuspended in DMEM/F12/10% FBS and washed with PBS, pH 7.3, 10% FBS. The cells were labeled with the primary (Oct4: Santa Cruz Biotechnology, Dallas, TX, USA; Nestin: Acris, Herford, Germany; Pax-6: Santa Cruz Biotechnology; Frizzled9, FZD9/CD349: BioLegend, San Diego, CA, USA; Musashi1: Merck Millipore, Billerica, MA, USA) and secondary antibodies (anti-mouse IgG Alexa Fluor 488; anti-rabbit IgG Alexa Fluor 488; anti-goat IgG FITC, all Life Technologies; anti-mouse IgM FITC, Merck Millipore) for 1 hour at 4°C each and washed 3 times with FACS buffer (1% FBS in PBS) between incubation steps. For intracellular FACS staining, the cells were initially fixed with 1% paraformaldehyde (PFA) in PBS for 10 min and blocked for unspecific binding by incubation with FACS buffer for 30 min. Cells were permeabilized with 0.1% Triton™ X-100 for 10 min and washed 3 times with FACS buffer before the incubation with the primary antibody. After incubation with the secondary antibody, the cells were washed 3 times, resuspended in the FACS buffer and the FACS analysis was performed. For extracellular FACS staining, the same procedure was followed excluding the PFA fixation step. Negative controls were obtained by incubating MSC with the secondary antibody only. Cells were analyzed by SORP LSR-II (BD

Biosciences) and quantified with flow cytometry analysis software (FlowJo v10, Tree Star, Inc., Ashland, OR, USA).

qRT-PCR

Total RNA was extracted from MSC at passage 5 using the QIAshredder (Qiagen, Venlo, The Netherlands) and RNeasy Mini Kit (Qiagen). cDNA was synthesized from 5µg of total RNA using SuperScript™ III reverse transcriptase (Life Technologies). The following primers and probes were used: OCT4/POU5F1: forward primer (fw) 5'-ACCCACACTGCAGCAGATCA-3', reverse primer (rv) 5'-CACACTCGGACCACATCCTTCT-3', probe (pr) 5'-CCACATCGCCCAGCAGCTTGG-TAMRA-3'; PAX6: fw 5'-GCTTCACCATGGCAAATAACC-3', rv 5'-GGCAGCATGCAGGAGTATGA-3', pr 5'-CCTATGCAACCCCCAGTCCCCAG-TAMRA-3'; MSI: fw 5'-CTCCAAAACAATTGACCCTAAGGT-3', rv 5'-GACAGCCCCCCCACAAAG-3', pr; 5'-CGAGCACAGCCCAAGATGGTGACTC-TAMRA-3'. TaqMan gene expression assays (Life Technologies) were used for NES (Hs00707120_s1) and FZD9 (Hs00268954_s1). Standard settings were used for qRT-PCR (7300 Real Time PCR System, Life Technologies; 45 cycles). The transcripts were normalized to a reference gene (GAPDH: fw 5'-GCTCCTCCTGTTCGACAGTCA-3', rv 5'-ACCTTCCCCATGGTGTCTGA-3', pr 5'-CGTCGCCAGCCGAGCCACA-TAMRA-3') with human fetal brain RNA as the calibrator.

Differentiation

MSC were differentiated into adipocytes, osteocytes and chondrocytes (StemPro differentiation kits, Life Technologies) and analyzed as described (Portmann-Lanz et al., 2006). Differentiation of MSC into oligodendrocyte progenitor (OPC)-like cells was done

as follows (Fu et al., 2007; Zhang et al., 2010): The MSC (passage 5) were trypsinized (0.25% trypsin, 1 mmol/l EDTA, Life Technologies) and replated (1:2 ratio) in DMEM/F12 medium containing 10 ng/ml epidermal growth factor (EGF, BD Biosciences, Franklin Lakes, NJ, USA) and N2 supplement (1:100, Life Technologies) for 3 days. Thereafter, $1x10^5$ MSC/ml were plated into ultra-low attachment cell culture flasks (Corning, Corning, NY) in neurospheres (NS) medium (neurobasal medium, 20 ng/ml bFGF, 20 ng/ml EGF, B27 1:50, Life Technologies) that lead to the formation of free-floating neurospheres. After three days in NS medium, neurospheres were plated on poly-L-lysine- and laminin- (Sigma-Aldrich) coated Lab-Tek® glass chamber slides (Sigma-Aldrich) for further differentiation (neurobasal medium, 10 ng/ml bFGF; 10 ng/ml PDGF; 1% FBS; 1 μM purmorphamine, Calbiochem, San Diego, CA).

Immunocytochemistry

Before immunostaining, the cells were fixed with 4% paraformaldehyde in PBS (pH 7.3, 10 min, room temperature (RT)) and treated with 0.1% Triton-X (in PBS, 10 min, RT; only for intracellular staining). The cells were then stained with the primary (PDGF-Rα: rabbit, 1:500, Abcam, Cambridge, UK; O4: mouse, 1:100, Merck Millipore; NF200: rabbit, 1:500, Acris; O1: mouse, 1:100, Merck Millipore; GFAP: mouse, 1:100, Merck Millipore; Olig2: goat, 1.200, Santa Cruz; Vimentin: mouse, 1:500, Sigma-Aldrich) and secondary (anti-mouse IgG, Alexa Fluor-488/594, 1:200; anti-rabbit IgG, Alexa Fluor-488/594, 1:200; anti-mouse IgM, Alexa Fluor-594, 1:200, all Life Technologies; anti-goat IgM, FITC, 1:200, Jackson ImmunoResearch, West Grove, PA) antibodies for 1 hour each, followed by three washes (PBS, 10 min) after each antibody. Fluorescein isothiocyanate-conjugated phalloidin (1:500, Sigma-Aldrich) was used to visualize actin

filaments. After staining, the cells were washed and visualized by fluorescence microscopy (Leica Microsystems, Wetzlar, Germany). The surface area of MSC was analyzed with the ImageJ software (Rasband WS. U.S. National Institutes of Health, Bethesda, MD, USA) after scale bar calibration.

Quantitative measurement of proteins secreted by the MSC

$1x10^5$ MSC grown in SM or SRM conditions were cultured with 2ml of medium in a 6-well plate. The media were collected after 48 hours and growth factors, chemokines and cytokines released from the cells quantified using Bio-Rad Luminex 100 Bio-Plex Liquid Array Multiplexing System. The following assays were performed: Human cytokine 27-plex panel (Cat. #M50-0KCAF0Y), human cancer biomarker panel 1, 16-Plex (Cat. #171-AC500M) and human cytokine SDF-1α set (Cat. #171-B6019M). Fresh medium was used as a negative control and the value was subtracted with SM and SRM condition to avoid any background signal present before analyzing the actual measurement.

Cell proliferation and wound healing assays

PrestoBlue™ cell viability reagent (Life Technologies) was used to quantitatively measure cell proliferation. Cells were harvested and the cell proliferation was analyzed at 0, 6, 12, 24 and 48 hours after onset of cultivation. To analyze the cell migration, MSC (SM or SRM) were grown to confluency. A sterile 200 µl pipette-tip was used to mimic a wound by scratching and removing a discrete area of the monolayer. Then, the plate was washed gently with PBS and the cells were cultured in their respective medium without serum (to prevent cell-proliferation). Cell migration at the wound site was observed at different time points up to 60 hours with time-lapse live imaging

(BioStation system, Nikon, Tokyo, Japan). The wound area was analyzed at different time points using ImageJ software.

Statistics

Statistical analysis was performed by analysis of variance (ANOVA) with Sigma plot™ 11.0 software (Systat Software, Inc., Chicago, IL). All experiments were conducted at least three times and the following symbols were used to show the degree of significance: "*" if P≤0.05, "**" if P≤0.01 and "***" if P≤0.001, respectively.

Results

Isolation of MSC and culture under SRM and SM condition

MSC grown in SRM condition showed different cell morphology when compared to cells grown in SM condition, as assessed by staining the cytoplasmic filaments vimentin and actin (Fig.1A). SRM MSC are smaller ($0.93x10^{-3}$ mm^2; SEM: $0.09x10^{-3}$ mm^2), more elongated and with more compact cytoplasm compared to the SM MSC ($5.12x10^{-3}mm^2$; SEM: $0.61x10^{-3}mm^2$) (Fig. 1A). Independent of the culture condition, cells passed the minimal criteria defined for multipotent mesenchymal stromal cells. The cells were highly positive (90%) for MSC markers (CD105, CD90, CD73), negative (≤ 2% positive cells) for hematopoietic and major histocompatibility complex markers (CD45, CD34, CD14, HLA-DR, figure not shown).

Effect of SRM condition on MSC marker expression

Flowcytometry indicated that in both conditions only few cells (< 1%) were positive for early stem cell markers such as Oct4 and PAX6 (Fig. 1B). In SRM MSC, nestin- (1.5-fold), musashi1- (1.7-fold) and FZD9-positive cells (12-fold) were significantly increased

when compared to the SM MSC (Fig. 1B). Gene expression profiles of MSC disclosed no significant differences of the mRNA levels of Oct4, PAX6 and FZD9 between the SRM and the SM conditions. Conversely, nestin (2.4-fold) and musashi1 (2.3-fold) were significantly upregulated in SRM MSC compared to SM MSC (Fig. 1C).

SRM condition promotes cell proliferation and migration

MSC had a higher proliferation rate when grown in the SRM compared to the SM condition. Both conditions resulted in equal levels up to 6 hours of culture, but SRM MSC proliferation was two-fold at 48 hours when compared to the SM MSC (Fig. 2A). In the cell migration and motility assay, MSC cultured in SRM were able to cover the wound area faster than the SM MSC (Fig. 2B, supplemental fig. 1). Moreover, the migration or the wound healing capability of the SRM MSC was significantly improved starting from 15 hours (SRM: 46.36%±2.72, SM: 29.37%±5.45) after scratching, compared to SM. The SRM MSC reached complete wound gap closure within 50 hours (SRM: 100%±0.0, SM: 58.16%±8.92), whereas SM MSC covered approximately only 50% of the wound area even after 60 hours (Fig. 2B).

SRM condition promote autocrine secretion of growth factors and cytokines

MSC grown in gelatin-coating/serum-free (SRM) conditions secreted significantly higher levels of IL-6 (4.0-fold), VEGF (2.5-fold), IL-8 (12.8-fold), SDF-1α (2.1-fold), IFN-γ (1.5-fold), G-CSF (3.3-fold), IL-12 (1.3-fold), RANTES (2.2-fold), IL-1ra (1.6-fold), eotaxin (1.2-fold), PDGF-BB (2.0-fold), TNF-α (1.4-fold), IL-9 (2.0-fold), IL-1b (40.1-fold), IL-13 (1.4-fold), when compared to MSC grown in SM condition. The following molecules were secreted in both conditions, but with no significant differences: HGF, sEGFR, sVEGFR-1, IP-10, Leptin, sHER2/neu, MCP-1, sVEGFR-2, Prolactin, PECAM-1,

Osteopontin, sTIE-2, PDGF-AB/BB, SCF, sIL-6Ra, GM-CSF, IL-17, IL-7, IL-15 and IL-5 (Fig. 2C, Supplemental table 1).

***In vitro* differentiation**

Multi-lineage differentiation (adipogenesis, osteogenesis and chondrogenesis) potential of both SM and SRM MSC was confirmed (Fig. 3A). After differentiation of neurospheres towards the oligodendroglial lineage, respective markers were assessed by immunofluorescence. After differentiation, cells from the SRM condition had a higher amount of cells positive for PDGFRα (2.2-fold), GFAP (2.3-fold), and Olig2 (1.1-fold) compared to SM (Fig. 3B & 3C).

Discussion

We found that preconditioning of the chorion-derived MSC improves the stemness, cell-proliferation, wound healing, cell-migration, secretome and enhances their innate neurogenic potential.

Comparison of the cell morphology and the proliferation of the SM and SRM MSC indicate that SRM MSC have a smaller cell size and higher proliferation rate than SM MSC. The intermediate filament vimentin acts as an organizer of a number of proteins involved in attachment, migration, and cell signaling (Ivaska et al., 2007). Although vimentin (Fig. 1A) is expressed in both conditions, the SRM MSC migrate better than the SM MSC (Fig. 2B), which is possibly the result of the compact arrangement of actin-filaments (Ananthakrishnan and Ehrlicher, 2007) and the secreted migratory cytokines (Fig. 2C). In accordance with published data, the early (progenitor) neural markers MSI1

and FZD9 are expressed by a higher proportion of cells grown in SRM when compared to the SM conditions (Fig.1B, 1C) (Kaneko et al., 2000; Park et al., 2010; Van Raay et al., 2001). Previous studies have shown that under serum deprivation, MSC express ESC and NSC markers such as nestin (Battula et al., 2007; Sauerzweig S, 2009). This indicates that SRM MSC are less differentiated and have potent proliferation and differentiation capacities (Battula et al., 2007) that could be beneficial for stem cell therapy. Possible reasons for the difference in the FZD9 protein and gene expression in SRM are that FACS only gives the percentage of FZD9+ cells, whereas the gene expression is the result of the total cell population. In addition, differences in protein concentration are only 20%-40% attributable to the variable mRNA levels due to the post-transcriptional regulation (Brockmann et al., 2007).

The SRM preconditioning of the chorion-derived MSC leads to a high protein expression of frizzled-9 (FZD9). Frizzled proteins are a family of transmembrane protein receptors that are activated by the Wnt signaling pathway that plays an important role in stem cell renewal, fate decision and early developmental stages (Ling et al., 2009). Stem cells express several types of frizzled receptors, which bind to different Wnt ligands, but FZD9 is highly expressed in neural precursor cells, the nervous system and in the developing brain (Van Raay et al., 2001), and it could possibly act as an essential marker for primitive MSC suitable for neuroregeneration.

The secretion of cytokines and growth factors (Fig. 2C) could play important roles in survival, proliferation, migration, homing and the stem cell fate. IL-6 and RANTES play roles in stem cell proliferation and maintenance (Pricola et al., 2009; Rice and Scolding, 2010). IFN-γ and eotaxin have important functions in neurogenesis and

oligodendrogenesis (Butovsky et al., 2006; Maysami et al., 2006). IL-13, VEGF and G-CSF have neuroprotective properties (Lu CZ, 2006; Rossi et al., 2011; Zheng et al., 2012). PDGF-bb promotes neural progenitor cell proliferation (Spassky N et al., 2001). Cytokines like IL-12, IL-1ra and IL-1b aid in the control of inflammation (Klassen et al., 2003; Robertson et al., 2002; Shin et al., 2011). In our experiments, MSC cultured in the SRM conditions acquired increased migratory properties. The cytokines IL-9, IL-8, SDF-1α and TNF- α have been reported to promote the migration and recruitment of neural stem cells (Klassen et al., 2003; Ni et al., 2004; Rice and Scolding, 2010; Weiss et al., 2010; Zhou et al., 2011). Thus, chorion-derived SRM MSC secrete essential factors that could enhance the repair mechanism by the recruitment of stem cells from nearby niches and the release of anti-inflammatory and growth factors. We further confirmed the capacity of chorion-derived MSC to differentiate towards OPC-like cells, shown by the strong expression of PDGFRα (Spassky N et al., 2001), GFAP (Sauerzweig S, 2009) and Olig2 (Marshall, 2005Marshall, 2005) markers (Fig. 3B & 3C).

Conflicts of interest: None declared.

Authors Contribution: R Periasamy designed and performed the experiments, analyzed the data, and wrote the manuscript. A Schoeberlein & D Surbek were responsible for authorization, instruction and revision of the manuscript. All authors approved the final version of the manuscript.

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References

Ananthakrishnan, R., Ehrlicher, A., 2007. The forces behind cell movement. Int J Biol Sci 3, 303-317.
Battula, V.L., Bareiss, P.M., Treml, S., Conrad, S., Albert, I., Hojak, S., Abele, H., Schewe, B., Just, L., Skutella, T., Bühring, H.-J., 2007. Human placenta and bone marrow derived MSC cultured in serum-free, b-FGF-containing medium express cell surface frizzled-9 and SSEA-4 and give rise to multilineage differentiation. Differentiation 75, 279-291.
Battula, V.L., Treml, S., Abele, H., Buhring, H.J., 2008. Prospective isolation and characterization of mesenchymal stem cells from human placenta using a frizzled-9-specific monoclonal antibody. Differentiation 76, 326-336.
Brockmann, R., Beyer, A., Heinisch, J.J., Wilhelm, T., 2007. Posttranscriptional expression regulation: what determines translation rates? PLoS Comput Biol 3, e57.
Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A.E., Pluchino, S., Martino, G., Schwartz, M., 2006. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cell Neurosci 31, 149-160.
Fu, S.L., Hu, J.G., Li, Y., Wang, Y.X., Jin, J.Q., Xui, X.M., Lu, P.H., 2007. A simplified method for generating oligodendrocyte progenitor cells from neural precursor cells isolated from the E16 rat spinal cord. Acta Neurobiol Exp (Wars) 67, 367-377.
Ivaska, J., Pallari, H.M., Nevo, J., Eriksson, J.E., 2007. Novel functions of vimentin in cell adhesion, migration, and signaling. Exp Cell Res 313, 2050-2062.
Kaneko, Y., Sakakibara, S., Imai, T., Suzuki, A., Nakamura, Y., Sawamoto, K., Ogawa, Y., Toyama, Y., Miyata, T., Okano, H., 2000. Musashi1: an evolutionally conserved marker for CNS progenitor cells including neural stem cells. Dev Neurosci 22, 139-153.
Klassen, H.J., Imfeld, K.L., Kirov, I.I., Tai, L., Gage, F.H., Young, M.J., Berman, M.A., 2003. Expression of cytokines by multipotent neural progenitor cells. Cytokine 22, 101-106.
Ling, L., Nurcombe, V., Cool, S.M., 2009. Wnt signaling controls the fate of mesenchymal stem cells. Gene 433, 1-7.
Lu CZ, X.B., 2006. G-CSF and neuroprotection a therapeutic perspective in cerebral ischaemia. Biochem Soc Trans 34, 1327-1333.
Marshall, C.A.G., 2005. Olig2 Directs Astrocyte and Oligodendrocyte Formation in Postnatal Subventricular Zone Cells. Journal of Neuroscience 25, 7289-7298.
Maysami, S., Nguyen, D., Zobel, F., Heine, S., Höpfner, M., Stangel, M., 2006. Oligodendrocyte precursor cells express a functional chemokine receptor CCR3: Implications for myelination. Journal of Neuroimmunology 178, 17-23.
Ni, H.T., Hu, S., Sheng, W.S., Olson, J.M., Cheeran, M.C., Chan, A.S., Lokensgard, J.R., Peterson, P.K., 2004. High-level expression of functional chemokine receptor CXCR4 on human neural precursor cells. Brain Res Dev Brain Res 152, 159-169.
Park, D., Xiang, A.P., Mao, F.F., Zhang, L., Di, C.-G., Liu, X.-M., Shao, Y., Ma, B.-F., Lee, J.-H., Ha, K.-S., Walton, N., Lahn, B.T., 2010. Nestin Is Required for the Proper Self-Renewal of Neural Stem Cells. Stem Cells 28, 2162-2171.

Portmann-Lanz, C.B., Baumann, M.U., Mueller, M., Wagner, A.M., Weiss, S., Haller, O., Sager, R., Reinhart, U., Surbek, D.V., 2010a. Neurogenic characteristics of placental stem cells in preeclampsia. Am J Obstet Gynecol 203, 399.e391-397.
Portmann-Lanz, C.B., Schoeberlein, A., Huber, A., Sager, R., Malek, A., Holzgreve, W., Surbek, D.V., 2006. Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. Am J Obstet Gynecol 194, 664-673.
Portmann-Lanz, C.B., Schoeberlein, A., Portmann, R., Mohr, S., Rollini, P., Sager, R., Surbek, D.V., 2010. Turning placenta into brain: placental mesenchymal stem cells differentiate into neurons and oligodendrocytes. Am J Obstet Gynecol 202, 294.e291-294.e211.
Pricola, K.L., Kuhn, N.Z., Haleem-Smith, H., Song, Y., Tuan, R.S., 2009. Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. J Cell Biochem 108, 577-588.
Rice, C.M., Scolding, N.J., 2010. Adult human mesenchymal cells proliferate and migrate in response to chemokines expressed in demyelination. Cell Adhes Migr 4, 235-240.
Robertson, M.J., Pelloso, D., Abonour, R., Hromas, R.A., Nelson, R.P., Jr., Wood, L., Cornetta, K., 2002. Interleukin 12 immunotherapy after autologous stem cell transplantation for hematological malignancies. Clin Cancer Res 8, 3383-3393.
Rossi, S., Mancino, R., Bergami, A., Mori, F., Castelli, M., De Chiara, V., Studer, V., Mataluni, G., Sancesario, G., Parisi, V., Kusayanagi, H., Bernardi, G., Nucci, C., Bernardini, S., Martino, G., Furlan, R., Centonze, D., 2011. Potential role of IL-13 in neuroprotection and cortical excitability regulation in multiple sclerosis. Mult Scler 17, 1301-1312.
Sauerzweig S, M.T., Lessmann V, Reymann KG, Braun H., 2009. A population of serumdeprivation-induced bone marrow stem cells (SD-BMSC) expresses marker typical for embryonic and neural stem cells. EXPERIMENTAL CELL RESEARCH 315 (2009) 50–66 315, 50–66.
Schoeberlein, A., Mueller, M., Reinhart, U., Sager, R., Messerli, M., Surbek, D.V., 2011. Homing of placenta-derived mesenchymal stem cells after perinatal intracerebral transplantation in a rat model. American Journal of Obstetrics and Gynecology 205, 277.e271-277.e276.
Shin, H.J., Baker, J., Leveson-Gower, D.B., Smith, A.T., Sega, E.I., Negrin, R.S., 2011. Rapamycin and IL-2 reduce lethal acute graft-versus-host disease associated with increased expansion of donor type CD4+CD25+Foxp3+ regulatory T cells. Blood 118, 2342-2350.
Spassky N, Heydon K, Mangatal A, Jankovski A, Olivier C, Queraud-Lesaux F, Goujet-Zalc C, Thomas JL, B., Z., 2001. Sonic hedgehog-dependent emergence of oligodendrocytes in the telencephalon: evidence for a source of oligodendrocytes in the olfactory bulb that is independent of PDGFRalpha signaling. Development 128, 4993-5004.
Ullah, Imran, Raghavendra Baregundi Subbarao, and Gyu Jin Rho. “Human Mesenchymal Stem Cells - Current Trends and Future Prospective.” Bioscience Reports 35.2 (2015): e00191.

Van Raay, T., Wang, Y.-K., Stark, M., Rasmussen, J., Francke, U., Vetter, M., Rao, M., 2001. frizzled 9 is expressed in neural precursor cells in the developing neural tube. Development Genes and Evolution 211, 453-457.
Weiss, N., Deboux, C., Chaverot, N., Miller, F., Baron-Van Evercooren, A., Couraud, P.O., Cazaubon, S., 2010. IL8 and CXCL13 are potent chemokines for the recruitment of human neural precursor cells across brain endothelial cells. J Neuroimmunol 223, 131-134.
Xu C, Inokuma M S, Denham J, Golds K, Kundu P, Gold J.D, M.K., C., 2001. Feeder-free growth of undif-ferentiated human embryonic stem cells. Nat Biotechnol 19, 971–974.
Zhang, H.-T., Fan, J., Cai, Y.-Q., Zhao, S.-J., Xue, S., Lin, J.-H., Jiang, X.-D., Xu, R.-X., 2010. Human Wharton's jelly cells can be induced to differentiate into growth factor-secreting oligodendrocyte progenitor-like cells. Differentiation 79, 15-20.
Zheng, X.R., Zhang, S.S., Yin, F., Tang, J.L., Yang, Y.J., Wang, X., Zhong, L., 2012. Neuroprotection of VEGF-expression neural stem cells in neonatal cerebral palsy rats. Behav Brain Res 230, 108-115.
Zhou, Y., Sonobe, Y., Akahori, T., Jin, S., Kawanokuchi, J., Noda, M., Iwakura, Y., Mizuno, T., Suzumura, A., 2011. IL-9 promotes Th17 cell migration into the central nervous system via CC chemokine ligand-20 produced by astrocytes. J Immunol 186, 4415-4421.

Figure captions

Figure 1. (A) Human chorion-derived MSC grown in no gelatin / serum (SM) condition (upper panel) or gelatin / serum-replacement (SRM) condition (lower panel), stained with DAPI (nuclei, blue), phalloidin (actin filaments, green) and vimentin (red). (B) Analysis of early stem cell and (pre-) neural markers analyzed by FACS in MSC cultured in SRM or SM condition (n=4). (C) Fold change of mRNA transcripts as measured by qRT-PCR (n=5). Mean ± SD, * P≤ 0.05, ** P≤ 0.01.

Figure 2. (A) Analysis of cell proliferation (SRM and SM MSC) using the PrestoBlue™ assay. (B) The percentage of the wound gap coverage by the SRM MSC compared to the SM MSC in the wound healing (migration) assay. (C) Secretion of cytokines, chemokines and growth factors analyzed by the Bio-Plex 100 system (BioRad, mean ± SD, n=4, * P≤0.05, ** P≤0.01, *** P≤0.001).

Figure 3. (A) Cytochemical analysis for the multilineage differentiation (adipogenic: Oil Red O for lipid droplets, osteogenic: BCIP/NBT for alkaline phosphatase activity, chondrogenic: toluidine blue O for sulfated glycosaminoglycans) of chorion MSC cultured under SM and SRM conditions. Undifferentiated MSC were used as control. Scale bars: 100µm, inset-scale bars: 20 µm (B) The neurospheres derived from SM and SRM conditions were differentiated into OPC-like cells and analyzed by

immunocytochemistry for PDGFRα (red), GFAP (green), and Olig2 (red) with nuclear stain DAPI (blue). Scale bars 100μm. (C) Graphs are presented as percentage of positive cells for PDGF-Rα, GFAP and Olig2 versus total cells (mean + SD, * P≤ 0.05 and *** P≤ 0.001).